Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
www.plantsjournal.com
JMPS 2024; 12(4): 105-110

Received: 06-05-2024
Accepted: 08-06-2024

Sourabh J Chougule
Students, Ashokrao Mane Institute of Pharmacy, Ambap, Kolhapur, Maharashtra, India

Seema Shinde
Assistant Professor, Ashokrao Mane Institute of Pharmacy, Ambap, Kolhapur, Maharashtra, India

Nilesh Chougule
Assistant Professor, Ashokrao Mane Institute of Pharmacy, Ambap, Maharashtra, India

Corresponding Author:
Sourabh J Chougule
Students, Ashokrao Mane Institute of Pharmacy, Ambap, Kolhapur, Maharashtra, India

Formulation and evaluation of polyherbal antifungal cream by using neem, guduchi and mint plant extract

Sourabh J Chougule, Seema Shinde and Nilesh Chougule

Abstract
Fungal infections pose a significant challenge to public health, necessitating the development of effective and safe antifungal agents. Herbal remedies have garnered attention for their potential antimicrobial properties, including antifungal activity. In this study, we formulated a polyherbal antifungal cream utilizing the synergistic effects of neem (*Tinospora cordifolia*), guduchi (*Tinospora cordifolia*), and mint (*Mentha* spp.). These botanical extracts are renowned for their broad-spectrum antimicrobial activities and have been traditionally used in various medicinal preparations. The cream was prepared using a standardized method and evaluated for its antifungal efficacy against clinically relevant fungal strains using agar diffusion and broth dilution methods. Additionally, physicochemical properties such as pH, viscosity, and stability were assessed to ensure product quality and consistency. Preliminary results demonstrated promising antifungal activity of the polyherbal cream against common fungal pathogens, including Candida albicans and dermatophytes. Furthermore, the cream exhibited favorable physicochemical characteristics suitable for topical application, including a pH conducive to skin health and optimal viscosity for easy Spreadability. Overall, our findings suggest that the polyherbal antifungal cream containing neem, guduchi, and mint holds great potential as a natural alternative for the management of fungal infections. Further studies including clinical trials are warranted to validate its efficacy and safety for clinical use.

Keywords: Fungal infection, antifungal activity, polyherbal cream, polyherbal formulation.

Introduction
As the body's largest organ, the skin serves as the primary defense against environmental stressors like dust, UV radiation, pathogens, and chemicals, which can lead to infections and aging. It also reflects aging and overall internal health [1]. The practice of using plants or plant parts to treat wounds or illnesses is called herbal medicine, botanical medicine, or herbalism [2]. This includes the use of seeds, leaves, stems, bark, roots, flowers, and extracts to create treatments such as topical applications, pills, capsules, teas, and tinctures. Many pharmaceutical drugs today are derived from these traditional remedies. This approach appeals to medical professionals due to its lower cost and generally safer use [3]. Medicinal plants are crucial for addressing serious illnesses globally, especially in developing countries where they are essential for basic healthcare needs. The medicinal value of these plants comes from chemically active compounds and secondary metabolites, like carbohydrates, alkaloids, glycosides, tannins, flavonoids, and phenolic compounds. Rising antibiotic resistance has made it increasingly important to explore plant-based natural products and secondary metabolites for new antimicrobial activities and unique mechanisms of action. Natural remedies for infectious diseases are effective and minimize the harmful side effects often seen with synthetic antimicrobials. Therefore, it's vital to study plant metabolites to confirm their traditional medicinal uses and identify active ingredients through chemical analysis [4]. Polyherbal compositions involve combining two or more plants. Ayurveda, through "Sarangdhar Samhita," discusses the concept of mutualism that underpins polyherbal remedies. While single-plant formulations contain active phytoconstituents, their quantities are often insufficient for therapeutic effects. Research has demonstrated that combining plants with varying potencies results in better outcomes due to synergistic effects, which can be pharmacokinetic or pharmacodynamic [5]. Creams are semisolid emulsions for external application, classified as water-in-oil (w/o) or oil-in-water (o/w) [6]. Cosmetics, especially those made from natural herbs, are effective for improving skin health by hydrating,

nourishing, and moisturizing the skin. This project aims to develop an antifungal herbal cream using a polyherbal combination of neem, guduchi, and mint [7].

Neem: Neem (*Tinospora cordifolia*) is a tree from the Meliaceae family, predominantly found in the Indian subcontinent. Historically, neem has been widely used to address various health issues. According to the World Health Organization, traditional medicine is universally practiced in developing nations. Specifically, in the Indian subcontinent, neem has served medicinal purposes for over 4500 years, utilizing its seeds, bark, and leaves. Though less commonly, its fruit, flowers, and roots are also employed. Neem leaf treats ailments like leprosy, eye disorders, nasal bleeding, intestinal worms, digestive problems, appetite loss, skin ulcers, cardiovascular diseases, fever, diabetes, gum disease, and liver issues. Additionally, neem leaves are used for inducing abortions and contraception. Topically, neem addresses skin conditions, wounds, ulcers, head lice, and serves as a mosquito repellent and skin softener. Furthermore, neem functions as an insecticide.

Table 1: Taxonomy Classification [8]

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	Azadirachta
Species	A. indica

Guduchi: *Tinospora cordifolia* (Willd.), also known as Guduchi or Giloy in Hindi, is a large, deciduous climbing shrub belonging to the Menispermaceae family. This glabrous plant is native to China and the tropical regions of the Indian subcontinent, where it can grow up to 300 meters high. The stem of *Tinospora cordifolia* is a key ingredient in several Ayurvedic medicines used to treat fever, dyspepsia, general debility, and urinary disorders. The stem acts as a diuretic, stomachic, and bile stimulant, while also providing benefits such as inducing constipation, relieving burning sensations and thirst, promoting vomiting, enriching the blood, and treating jaundice. Additionally, the stem extract is effective for various skin conditions. In combination with other medications, the root and stem of T. cordifolia are used as antidotes for snake bites and scorpion stings. The dry bark of T. cordifolia possesses antispasmodic, antipyretic, antiallergic, anti-inflammatory, and anti-leprotic properties.

Table 2: Taxonomy Classification: [9-14]

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Ranunculales
Family	Menispermaceae
Genus	Tinospora
Species	T. cordifolia

MINT: Mint, a fragrant herb from the Lamiaceae family, is rich in essential oils and widely used in both culinary and herbal applications. This family encompasses over 7,000 species within 250 genera. Mint plants have square stems, though their flower clusters vary. Some species are notable for having lacunar parenchyma tissue throughout. Research shows that Lamiaceae plants have medicinal benefits, including antifungal properties. Fungi, which can be saprophytic or facultative parasites, often live symbiotically with their hosts. They are significant to public health, as chronic fungal infections can affect the reproductive, renal, oral, and skin systems. Moreover, opportunistic fungal infections, such as those from Candida species, are commonly linked to metabolic disorders like diabetes.

Table 3: Taxonomy Classification: [15-19]

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	*Mentha* L
Species	Lamiales

Material and Instruments

Table 4: Instruments used for work

Sr. No.	Name Of Instrument
1.	Soxhlet Apparatus
2.	Electronic weighing balance
3.	pH meter
4.	Brookfield viscometer
5.	Heating mantle
6.	Electronic waterbath

Table 5: Chemicals used for work

Sr. No.	Chemicals
1.	Ethanol
2.	Clove oil
3.	Cinnamon oil
4.	Stearic acid
5.	Cetyl alcohol
6.	Potassium hydroxide
7.	Glycerine
8.	Propyl paraben
9.	Methyl paraben
10.	Distilled water

Extraction

Neem, Guduchi, Mint ethanolic extract preparation.

- The fine powder was meticulously selected.
- It was filtered using a 43-mesh sieve and stored in an airtight container for future use.
- Using the hot extraction method with a Soxhlet apparatus, around 50 grams of the powdered material were extracted with ethanol as the solvent.
- The extraction process continued until the solvent in the thimble became clear, after which a few drops were collected in a test tube at the end of the cycle and tested for chemical composition.
- The extract was dried using a rotary vacuum evaporator after each extraction.
- Additionally, a portion of the extract was saved for preliminary phytochemical screening [20].

Preliminary Phytochemical Investigation

The ethanolic extract underwent qualitative chemical analysis. Various procedures were employed to detect the presence of different phytochemical compounds in the extract. Key bioactive components in plants include steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, saponins, and glycosides. Phytochemicals serve as templates for lead

optimization programs aimed at developing safe and effective drugs. The following procedures were used to identify the various chemical constituents in the extract.

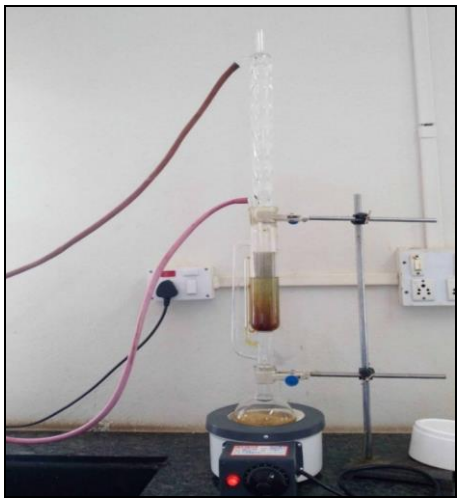

Fig 1: Process of extraction using Soxhlet apparatus

Test

Test for Saponins

- **Foam test:** Place a small extract in a test tube with a small amount of water. Shake it vigorously. If foam remains for 10 minutes, it indicates the presence of saponin.

Test for Alkaloids

- **Mayer's test:** 2-3 ml of the filtrate, when mixed with a small amount of Mayer's reagent, forms a precipitate.
- **Wagner's test**: When 2-3 ml of the filtrate is mixed with a small amount of Wagner's reagent, it produces a reddish-brown color.

Test for Tannins

- **Ferric chloride test:** Add a few drops of neutral ferric chloride solution to the alcoholic extract. The development of a green color indicates the presence of tannins.

Test for Steroids

- **Liebermann's reaction:** Mix 3 milliliters of the extract with an equal amount of acetic anhydride. Heat the mixture and allow it to cool. Introduce a small amount of concentrated sulfuric acid. This process results in the appearance of a blue color.

Test for Flavanoids

- **Alkaline reagent test:** When the test solution reacts with sodium hydroxide, it exhibits a heightened yellow coloration, which reverses to colorlessness upon the addition of a small amount of dilute acid.

Test for Terpenoids

- **Salkowski reaction:** Mix 2 ml of the extract with 2 ml of chloroform and 2 ml of concentrated sulfuric acid. Shake the mixture thoroughly. The chloroform layer will turn red, while the acid layer will exhibit a fluorescence that appears greenish-yellow.

Test for reducing sugar

- **Benedict's test:** Combine equal amounts of Benedict's reagent and the test extract in a test tube. Heat the mixture in a boiling water bath for 5 minutes. The color of the solution changes to green, yellow, or red, indicating the quantity of reducing sugars in the test solution.

Test for proteins

- **Biuret test:** Mix 2ml of Biuret reagent with 2ml of the extract, ensuring thorough shaking, followed by gentle warming on a water bath. The development of a red or violet color indicates the presence of proteins. To 3ml of the extract, add 4% NaOH and a few drops of 1% CuSO4 solution. A violet or pink color will appear [21].

Preformulation study

Preformulation studies are essential to guarantee the creation of a stable, effective, and safe dosage form. This stage involves pharmacists analyzing the physical and chemical properties of drug substances and their interactions with different formulation components. The objectives of Preformulation studies include

- Identifying the essential physicochemical parameters of a new drug substance and
- Assessing its compatibility with formulation excipients.

Experimental Design:

Formulation of Herbal Cream

Preparation of herbal Cream

1. Selection of excipients

Neem, Guduchi, Mint is collected from the ayurvedic shop from Kolhapur. The raw materials and chemicals were taken from Ashokrao mane institute of pharmacy, ambap, kolhapur. All ingredients and excipients used are given in the Table.

2. Method of preparation

A water-in-oil emulsion cream was developed by formulating an emollient mixture starting with stearic acid, which melts at 75°C. Cetyl alcohol was then added and allowed to dissolve, followed by the incorporation of clove oil and cinnamon oil. This mixture constituted the oil phase (Part A) of the emulsion. Concurrently, the water phase (Part B) was prepared by dissolving Neem, Guduchi, and Mint ethanolic extracts, along with KOH solution, glycerine, methyl paraben, and propyl paraben in water, heated to 75°C. The oil phase was then poured into the mortar immediately after heating, and the water phase was added gradually to the oil phase while continuously stirring with a pestle. The cooling process facilitated the formation of the cream formulation. Detailed formulation ingredients (A1, A2, A3) and their respective quantities are provided in Table [22].

Table 6: Formulation table

Sr. No.	Ingredients	Batches			Role of ingredient
		A1	A2	A3	
1	Ethanolic extract	0.5 gm	0.5 gm	0.5 gm	Therapeutic agent
2	Clove oil	1 ml	1 ml	1 ml	Active ingredient
3	Cinnamon oil	1 ml	1 ml	1 ml	Active ingredient
4	Stearic acid	5 gm	5 gm	5 gm	Emollient

5	Cetyl alcohol	1.6 gm	1.6 gm	1.6 gm	Emollient, Co-emulsifier
6	Potassium hydroxide	0.5 gm	0.5 gm	0.5 gm	Alkali reagent, Emulsifier
7	Glycerine	15 ml	15 ml	15 ml	Moistening reagent
8	Propyl paraben	0.2 gm	0.2 gm	0.2 gm	Preservative
9	Methyl Paraben	0.2 gm	0.2 gm	0.2 gm	Preservative
10	Distilled water	QS	QS	QS	Vehicle

Evaluation of Cream

1. Physical Evaluation: Physical parameters such as color and appearance were evaluated.

2. Homogenecity: All formulated creams underwent homogeneity testing through visual inspection post-container settling, assessing their appearance and the absence of any aggregates.

3. pH: The pH of different cream formulations was assessed using a digital pH meter. Specifically, 2.5 grams of each cream sample was precisely weighed and dispersed in 25 mL of distilled water, allowing it to sit for two hours. pH measurements for each formulation were conducted three times, and the average values were reported. The pH of the dispersions was determined using a pH meter.

4. Spreadability: The Spreadability of creams was assessed using a wooden block apparatus equipped with a pulley at one end. This method measured the slip and drag characteristics of creams. Approximately 2 grams of the cream under study were placed on a fixed ground slide. Another glass slide of the same dimensions as the ground slide, equipped with a hook, was placed on top to sandwich the cream. A weight of 1 kg was applied to the top slide for 5 minutes to remove air and ensure a uniform cream film between the slides. Excess cream was removed from the edges. Subsequently, a 50 g weight was pulled with a string attached to the hook, and the time (in seconds) taken for the top slide to travel a distance of 6.5 cm was recorded. A shorter time interval indicated better Spreadability.

Spreadability was calculated using the following formula:
$S = M \times L / T$
Where, S = Spreadability,
M = Weight in the pan (tied to the upper slide),
L = Length moved by the glass slide and
T = Time (in sec.) taken to separate the slide completely each other.

5. Viscosity: The viscosity of the herbal cream was measured using a Brookfield rotational viscometer with spindle no. 64 at various speeds: 5, 10, 20, 30, and 50 rpm. Each measurement was taken after allowing the sample to reach equilibrium for two minutes. This procedure was repeated three times to ensure accuracy [23-26].

Antifungal activity *in vitro* techniques

1. Preparation of agar medium

1. Prepare nutrient agar by dissolving 2.8 grams of nutrient agar in 100 ml of distilled or deionized water.
2. Heat the mixture with regular stirring until the agar is completely dissolved. Sterilize the medium by autoclaving at 121°C for 15 minutes.
3. After sterilization, check the pH of each batch. The pH should be maintained between 7.2 and 7.4 at room temperature. This can be tested by mixing a small amount of medium with distilled water or allowing the medium to surround a pH meter electrode.
4. Allow the agar medium to cool to a temperature of 40-50 °C. Pour the agar into sterile glass or plastic petri dishes on a level surface to achieve a consistent depth of 4 mm.
5. Let the agar solidify.
6. Before use, dry the plates in an incubator set at 30-37 °C with lids slightly open for no longer than 30 minutes or until excess surface moisture evaporates. The medium should remain moist but free from water droplets on the surface. Presence of water droplets can lead to excessive bacterial growth, potentially compromising the accuracy of results and making them susceptible to contamination [27-33].

2. Agar well diffusion method

1. Initially, prepare the media, consisting of nutrient agar and nutrient broth.
2. Pour the agar into plates to cultivate the bacteria or organisms being tested.
3. Use the broth to culture the bacteria.
4. Once the media is prepared, inoculate 2-3 mL of Mueller-Hinton broth (MHB) with a loop of the tested bacteria, such as E. coli and S. aureus, and incubate at 37 degrees Celsius.
5. After 24 hours, remove the bacteria and adjust their turbidity to the McFarland standard (using barium chloride and sulfuric acid) to ensure a consistent bacterial density on each plate.
6. With the turbidity matched, spread the bacteria on nutrient agar plates using a cotton swab, rotating continuously at a 60-degree angle.
7. Once dried, create wells in the plates with a borer, then add the positive and negative controls, along with the extracts, to the wells.
8. Incubate the plates for 24 hours at 37 degrees Celsius, then observe them.
9. Measure the zone of inhibition after 24 hours, which indicates the extent to which the extracts inhibit bacterial growth [34].

Results and Discussion

Extraction of Neem, Guduchi and Mint.

Table 7: Extractive values of Neem, Guduchi, Mint

Sample	Extraction method	Solvent used	Wt. of sample	Extraction value (%w/w)
Neem, Guduchi, Mint Fine powder	Soxhlet extraction	Ethanol	30 gm	10% w/w

Physicochemical evaluation of cream

1. Physical Appearance

All formulation batches were found to be homogeneous Brown cream preparations.

Table 8: Physical appearance of cream

Sr. No.	Batch	Color	Appearance
1	A1	Light Brown	Brown
2	A2	Brown	Brown
3	A3	Dark Brown	Brown

2. Homogeneity

All developed creams were tested for homogeneity by visual inspection after the creams have been set in the container.

Table 10: Homogeneity of formulation

Sr. No.	Batch	Homogenecity
1	A1	Homogeneous
2	A2	Homogeneous
3	A3	Homogeneous

3. Measurement of pH

The pH values of all prepared formulation ranged from 6-7 which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

4. Spreadability

The time in seconds require to separate the two slides was taken as measure of Spreadability.

Table 11: pH and Spreadability of extracts formulation

Sr. No.	Batch	pH	Spreadability (gm.sm/sec)
1	A1	6.5 /±0.03	16.10/±0.005
2	A2	7.2/±0.03	15.52/±0.005
3	A3	7.5/±0.03	15.40/±0.005

5. Viscosity

Viscosity of cream was determined by using Brookfield rotational viscometer at 5, 10, 20, rpm. Each reading was taken after equilibrium of the sample at the end of two minutes. The samples were repeated three times.

Table 12: Viscosity value of herbal cream

Sr. No.	Rpm	Viscosity (Cps)
1	5	3610 ± 0.20
2	10	3714 ± 0.21
3	20	4020± 0.11

Table 13: Zone of Inhibition

Sr. No.	Details	Concn. (%)	Zone of Inhibition (*E coli*)
1	Fluconazole	100	10
2	Cream	100	20
3	Extract	10	8
4	Saline water	5	No zone

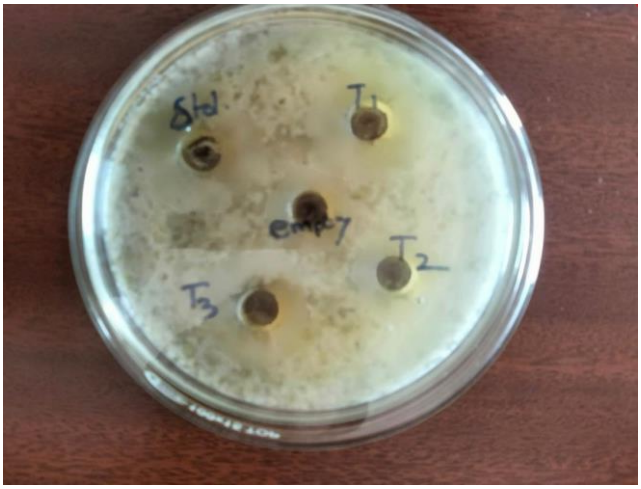

Fig 2: Zone of Inhibition

Conclusion

The in vitro evaluation of the polyherbal formulation comprising neem (*Tinospora cordifolia*), guduchi (*Tinospora cordifolia*), and mint (*Mentha* spp.) demonstrates promising antifungal activity against various fungal pathogens. The synergistic effects observed in this study underscore the potential of combining multiple herbal extracts to enhance antifungal efficacy. Neem, known for its broad-spectrum antimicrobial properties, exhibited strong antifungal effects, contributing significantly to the overall activity of the formulation. Guduchi, traditionally valued for its immunomodulatory and adaptogenic properties, also showed notable antifungal activity, reinforcing its role in the polyherbal mix. Mint, with its well-documented antimicrobial and anti-inflammatory properties, further enhanced the formulation's effectiveness against fungal pathogens. The results from the agar well diffusion method and minimum inhibitory concentration (MIC) assays indicated that the polyherbal formulation was effective in inhibiting the growth of common fungal strains, such as Candida albicans and Aspergillus niger. This suggests that the combined extracts have a broad spectrum of activity, potentially offering an alternative to conventional antifungal treatments.

Reference

1. Tzellos TG, Klagas I, Vahtsevanos K, Triaridis S, Printza A, Kyrgidis A, *et al*. Extrinsic ageing in the human skin is associated with alterations in the expression of hyaluronic acid and its metabolizing enzymes. Experimental Dermatology. 2009;18:1028-1035.
2. Anonymous. British Pharmacopoeia. Department of Health & Social Services for Northern Ireland. 1999;2:1583.
3. Ansel HC, Popovich NG, Allen LV. Pharmaceutical Dosage Forms and Drug Delivery Systems. 6th ed. New Delhi: B.I. Waverly Pvt. Ltd.; c1995, 240-245.
4. Thirumurugan K, Shihabudeen MS, Hansi PD. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. Steroids. 2010;1(7):430-434.
5. Tayade JA, Patil AV. Polyherbal formulation. Proceedings of the 3rd International Conference and Exhibition on Pharmacognosy, Phytochemistry & Natural Products. 2015;3(6):137.
6. Das T, Debnath J, Bipulnath, Dash S. Formulation and evaluation of an herbal cream for wound healing activity. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(suppl 2):693-697.
7. Pal A, Soni M, Patidar K. Formulation and evaluation of poly herbal cream. International Journal of Pharmaceutical and Biological Archives. 2014;5(4):67-71.
8. Kumar VS, Navaratnam V. Neem (*Tinospora cordifolia*): Prehistory to contemporary medicinal uses to humankind. Asian Pacific Journal of Tropical Biomedicine. 2013;3(7):505-514.
9. Anonymous. Wealth of India: A dictionary of Indian Raw Materials and Industrial Products. 1st ed. Vol. X. New Delhi: CSIR; 2003,251-252.
10. Vaidya DB. Materia Medica of Tibetan Medicine. Delhi: Sri Satguru Publications 1994, 163.
11. Bhandari C. Vanaushadhi Chandrodaya. 1st Ed. Varanasi: Chaukhamba Sanskrit Sansthan; 2006;3:86.
12. Nayampalli SS, Ainapure SS, Samant BD, Kudtarkar RG, Desai NK, Gupta KC, *et al*. A comparative study of diuretic effects of *Tinospora cordifolia* and hydrochlorothiazide in rats and a preliminary phase I

study in human volunteers. Journal of Postgraduate Medicine. 1988;34:233-236.
13. Aiyer KN, Kolammal M, editors. Pharmacognosy of Ayurvedic Drugs, Series 1. 1st Ed. Trivandrum: The Central Research Institute; c1963.
14. Raghunathan K, Mittra R, editors. Pharmacognosy of Indigenous Drugs. New Delhi: Central Council for Research in Ayurveda and Siddha; c1982.
15. Raja RR. Medicinally potential plants of Labiatae (Lamiaceae) family: an overview. Research Journal of Medicinal Plant. 2012;6(3):203-213.
16. Stankovic M, editor. Lamiaceae Species. MDPI; c2020.
17. Chaker AN, Boukhebti H, Sahli F, Haichour R, Sahraoui R. Morphological and anatomical study of two medicinal plants from genus *Mentha*. Advances in Environmental Biology. 2011;5(2 SPEC. ISSUE):219-221.
18. Denning DW, Hope WW. Therapy for fungal diseases: opportunities and priorities. Trends in Microbiology. 2010;18(5):195-204.
19. Lim SMS, Sinnollareddy M, Sime FB. Challenges in antifungal therapy in diabetes mellitus. Journal of Clinical Medicine. 2020;9(9):1-9.
20. Jamadar MJ, Shaikh RH. Preparation and evaluation of herbal gel formulation. Journal of Pharmaceutical Research & Education. 2017;1(2):201-224.
21. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 9th Ed. Pune: Nirali Prakashan; c2002, 149-160.
22. Sahu RK, Roy A, Kushwah P, Sahu A. Formulation and development of face cream containing natural products. Research Journal of Topical and Cosmetic Sciences. 2012;3(1):16-19.
23. Goyal S, Sharma P, Ramchandani V, Shrivastava SK, Dubey PK. Novel anti-inflammatory topical herbal gels containing *Withania somnifera* and *Boswellia serrata*. International Journal of Pharmaceutical and Biological Archives. 2011;2(4):1087-1094.
24. Mishra US, Murthy PN, Mishra D, Sahu K. Formulation and standardization of herbal gel containing methanolic extract of *Calophyllum inophyllum*. Asian Journal of Pharmaceutical and Clinical Research. 2011;1(1):276-289.
25. Dixit G, Misal G, Gulkari V, Upadhye K. Formulation and evaluation of polyherbal gel for anti-inflammatory activity. International Journal of Pharmaceutical Sciences and Research. 2013;4(3):1186-1191.
26. Mishra US, Murthy PN, Pasa G, Nayak RK. Formulation and evaluation of herbal gel containing methanolic extract of Ziziphus xylopyrus. International Journal of Biomedical and Pharmaceutical Research. 2011;1(4):207-218.
27. Balouiri M, Sadiki M, Ibnsouda SK. Antimicrobial activity testing methods. Chemist Notes. Available from: https://chemistnotes.com/biochemistry/antimicrobial-activity-by-agar-well-diffusion/.
28. Alderman DJ, Smith P. Development of draft protocols of standard reference methods for antimicrobial agent susceptibility testing of bacteria associated with fish diseases. Aquaculture. 2001;196:211-243.
29. Anonymous. Antimicrobial susceptibility testing: a system for standardisation. Becton Dickinson and Company, Hong Kong; c1986.
30. Bailey WR, Scott EG. Diagnostic Microbiology. 2nd ed. Japan: Toppan Company Ltd.; c1966, 257-270.
31. Finegold SM, Martin WJ. Bailey and Scott's Diagnostic Microbiology. 6th ed. London: The CV Mosby Company; c1982, 532-557.
32. NCCLS. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard. 2nd Ed. Wayne, Pennsylvania: NCCLS; 2002. NCCLS document M31-A2 (ISBN: 1-56238-461-9).
33. Prescott LM, Harley JP, Klein DA. Microbiology. 2nd ed. England: Wm C Brown Publishers; c1993. p. 325-343.
34. Antimicrobial activity by agar well diffusion method. Chemist Notes. Available from: https://chemistnotes.com/biochemistry/antimicrobial-activity-by-agar-well-diffusion/.